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The Role of Inflammation in Zinc, Amino Acid and Protein Alterations Induced by Zinc Chloride

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RUNNING TITLE: INFLAMMATION INDUCED BY IP $ZnCl_2$

CATEGORY: Pathology

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the author do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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Zinc therapy has been advocated by Chvapil to ameliorate certain aspects of selected inflammatory diseases based on the hypothesis that zinc contributes to the stability of membranes (1). Zinc sulfate given per os has in fact been used in patients to treat ulcers (2) and rheumatoid arthritis (3) with some indication of mild success. In rats, zinc chloride administered ip, is effective in muting the toxicity of endotoxin (4) or alcohol (5) when these agents are also administered ip. The protection afforded by zinc against the lethal effects of endotoxin appears to be limited to the case where endotoxin is administered ip and involves, in some as yet undefi[illegible]ner, reduction in the absorption of the toxin from the perit[illegible] cavity (4). We have also recently observed that concomitant ip injections of $ZnCl_2$ have a differential effect as regards the lethality of bacterial infections in rats, enhancing the mortality of murine typhoid but both slowing the onset of death and somewhat muting the mortality due to tularemia and pneumococcal sepsis (6).
In light of the above findings in rats and the potential use of zinc salts in the treatment of certain inflammatory diseases in patients, we thought it advisable to ascertain what are the effects of zinc salts themselves on the host. We directed our attention to the effects of ip injections of varying amounts of $ZnCl_2$ on amino acid and plasma protein metabolism because zinc has been implicated as a co-factor in the metabolism of certain amino acids and the synthesis of proteins (7, 8).

Materials and methods. Fisher-Dunning male rats, 200-225 g (Microbiological Associates, Walkersville, Md.) were used in all studies and were fasted after the injection of zinc chloride or, in the case of the controls, physiological saline. Zinc chloride (Fisher Scientific, Fairlawn, N.J.) was dissolved in sterile saline. Plasma and tissue zinc content was determined by atomic absorption spectrophotometry (9). The tissue samples (1 g) were solubilized by the addition of 1 ml of 25% tetramethylammonium hydroxide (10). To assess amino acid transport the rats were injected sc with 1 μCi [^{14}C]aminoisobutyric acid/100 g body weight 24 hr prior to the injection of zinc chloride or saline (11). Phenylalanine and tyrosine concentration in plasma were determined using a Beckman model 121 M amino acid analyzer (Beckman Instruments, Silver Spring, Md.). Plasma glucagon and insulin were measured by double antibody radioimmunoassay (12). Plasma seromucoid was determined according to the procecure of Neuhaus et al. (13); α_2-macrofetoprotein by radial immunoassay (14). Plasma β-glucuronidase acitivity was measured as described previously (4). Total white blood cell count was determined with a Coulter counter and the differential by counting 100 white cells in thin smears. Statistical significance was assessed by analysis of variance.

Results. The plasma zinc concentration at 5 hr after ip injection of $ZnCl_2$ is a curvilinear function of the dose administered with a significant increase versus controls at 8 and 16 mg/kg body weight (Fig. 1A). In contrast, hepatic zinc content is a linear function of the dose, while skeletal muscle content remains unchanged (Fig. 1B). Amino acid uptake by liver and muscle,

approximated by the [^{14}C]aminoisobutyrate (AIB) content of these tissues is depicted in Fig. 2. There was an increased uptake of ^{14}C-AIB into liver 5 hr after ip injection of either 8 or 16 mg/kg $ZnCl_2$. Muscle on the other hand displayed a slight, albeit statistically significant, decrease in ^{14}C-AIB content at 2, 4 and 16 mg/kg. The insert in the upper left corner of the figure indicates that there were no statistically significant alterations in peripheral plasma glucagon or insulin for any dose of zinc at 5 hr. Another evidence of altered amino acid distribution or metabolism is the plasma phenylalanine/tyrosine (P/T) ratio. There is a significant increase in the plasma P/T ratio at 4, 8, and 16 mg $ZnCl_2$ (Table I).

Evidence of tissue damage and/or inflammation 5 hr after the ip injection of varying amounts of $ZnCl_2$ are also presented in Table I. There is a significant increase in the percent neutrophils in the blood at 4-16 mg of $ZnCl_2$ but no change in total white blood cell count. There is an increase in plasma β-glucuronidase activity but only at the 4 mg/kg dose of $ZnCl_2$. There is histologic evidence of localized inflammation in 25% of the livers taken from animals given 8 mg and in 100% of the livers taken from animals given 16 mg $ZnCl_2$.

Twenty-four hours after the ip injection of varying amounts of $ZnCl_2$, plasma zinc concentrations was still significantly above control values in those animals given 8 or 16 mg (Table II). In contrast to 5 hr postinoculation, all rats receiving zinc displayed significant decreases in total white cell count, but only those which had received 8 or 16 mg zinc showed an increase in percent neutrophils. There was a small but significant increase in seromucoid concentration at 24 hr after 8 or 16 mg of $ZnCl_2$.

Alpha$_2$-macrofetoprotein was detectable in the plasma of those rats receiving 4-16 mg of $ZnCl_2$ with greater levels of this fetal globulin being found at the higher doses of zinc.

Discussion. Zinc salts are well known for their irritant properties. Thus, it is surprising that on the one hand zinc salts may, in certain well-defined instances, be of potential therapeutic value (2,3) and on the other that these irritant properties are overlooked in experiments involving the parenteral administration of zinc salts. This paper presents evidence that the ip injection of $ZnCl_2$ produces systemic manifestations of inflammation comprised of (but not necessarily limited to) neutrophilia, zinc and amino acid redistribution and alterations in plasma protein concentration.

Neutrophilia is not uncommon during infection and inflammation and can be produced in healthy animals by the injection of a factor (or factors) derived from leukocytes (15,16). A decrease in plasma zinc concentration and an increase in hepatic zinc content have been demonstrated during inflammation and infection (17,18) and also can be elicited by leukocytic derived factors (LEM) (19). The uptake of zinc by liver presumably involves the formation of metallothionein (20,21); there is evidence that hepatic synthesis metallothionein-like proteins can be induced by LEM (22). Increased amino acid transport into liver has been observed during a number of infections (11,18) and inflammation (17) and also can be elicited by the injection of LEM (23). An increase in the serum phenylalanine tyrosine ratio has been detected during a wide variety of infections in man and experimental animals and can be produced by the repeated injection of LEM (24-27). Increased plasma seromucoid concentration

and the dectectable presence of α_2-macrofetoprotein in the plasma occurs during inflammation and infection (17,18,26) and in response to LEM (28).

The ip injection of 4-16 mg of zinc chloride in sterile saline elicits all of the above reactions, neutrophilia, amino acid and zinc uptake by liver, increased plasma phenylalanine tyrosine ratio and both increased seromucoid concentration and the presence of α_2-macrofetoprotein in the plasma. The occurrence of all these systemic sequelae and indices of inflammation are consonant with the known irritant properties of zinc salts.

Moreover, the presence of hepatic capsulitis in those animals receiving the 8 and 16 mg doses of $ZnCl_2$ indicate localized inflammation. The significant increase in plasma β-glucuronidase activity at the 4-mg dose of $ZnCl_2$ is consistent with tissue damage, particularly liver (29). Considering the presence of capsulitis at 8 and 16 mg of $ZnCl_2$ the absence of additional increases in plasma β-glucuronidase is surprising, but considering the proposed role of zinc in stabilizing membranes (1) may not be totally unexpected. It is conceivable that although the higher concentrations of $ZnCl_2$ induce a greater degree of inflammation, they also reduce the release of certain lysosomal enzymes. Finally, although increases in glucagon and insulin have been implicated in the enhanced uptake of amino acids by liver produced by LEM (30) these hormones appear not to be operative in this instance, in that no significant alterations in either hormone are detectable at any of the doses of zinc chloride.

To what degree these metabolic and physiologic sequelae, as well as LEM itself, play a role in the protective (4,5,31-33) activities attributed to zinc remain to be assessed. For example, it has been proposed that certain of the metabolic alterations which do occur during infections, presumably a result of LEM, may be of benefit to the host (34). Also, it is not clear to what extent the induction of hepatic and intestinal metallothionein (21,35) or α-aminolevulinic acid dehyratase in red blood cells (36) is a direct effect of zinc itself or an indirect effect arising from the inflammatory properties of zinc salts. This is not to say that all of the consequences of injection of zinc salts can be ascribed to inflammation and are mediated by LEM, but rather that inflammation and LEM must be taken into consideration when zinc salts (or other potential irritants) are administered parenterally.

Summary

The ip injection of zinc chloride (4-16 mg/kg) produces a curvilinear increase in plasma zinc concentration, a linear increase in liver zinc content, and an increase in the hepatic uptake of [^{14}C]aminoisobutyrate which are significantly greater than control values. This dose range of $ZnCl_2$ also produces neutrophilia, increases in the plasma phenylalanine/tyrosine ratio and seromucoid and the appearance of α_2-macrofetoprotein in the plasma, all of which are indicative of inflammation. It is suggested that some of the protective and metabolic effects ascribed to zinc may in fact be the result, at least in part, of the inflammation which occurs when zinc salts are injected ip.

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TABLE 1

Evidences of inflammation at 5 hours

Mg $ZnCl_2$ kg body wt	Mean + SEM			
	Phenylalanine Tyrosine	% Neutrophils	β-glucuronidase µg/dl	Histology
0 (saline)	0.99 ±0.03	24 ± 2	63 ± 6	-
1	1.19 ±0.07	22 ± 3	57 ± 5	-
2	1.23 ±0.06	22 ± 2	64 ± 4	-
4	1.47 ±0.05**	39 ± 3**	101 ± 8**	-
8	1.70 ±0.05**	34 ± 2*	82 ± 2	hepatic capsulitis 2/8
16	1.72 ±0.03**	53 ± 2**	84 ± 6	hepatic capsulitis 6/6

*P < 0.01, **P < 0.001 vs saline.

TABLE 2

Evidence of inflammation at 24 hours

Mg $ZnCl_2$ kg body wt	Zinc µg/dl	Total WBC $x10^6/mm^3$	% Neutrophils	Seromucoid mg/dl	α_2-macrofetoglobulin units/ml
0	127 ± 3	7.4 ±0.6	15 ± 1	372 ± 19	0
1	135 ± 3	5.0 ±0.2**	17 ± 1	345 ± 24	0
2	148 ± 5	4.9 ±0.2**	16 ± 1	350 ± 11	0
4	153 ± 5	5.5 ±0.1**	15 ± 2	378 ± 10	1.4 ±0.2
8	173 ± 6*	5.5 ±0.4**	34 ± 2**	476 ± 15**	3.6 ±0.3
16	252 ± 24**	5.8 ±0.1*	38 ± 2**	478 ± 34**	6.2 ±1.5

*P < 0.01; **P < 0.001.

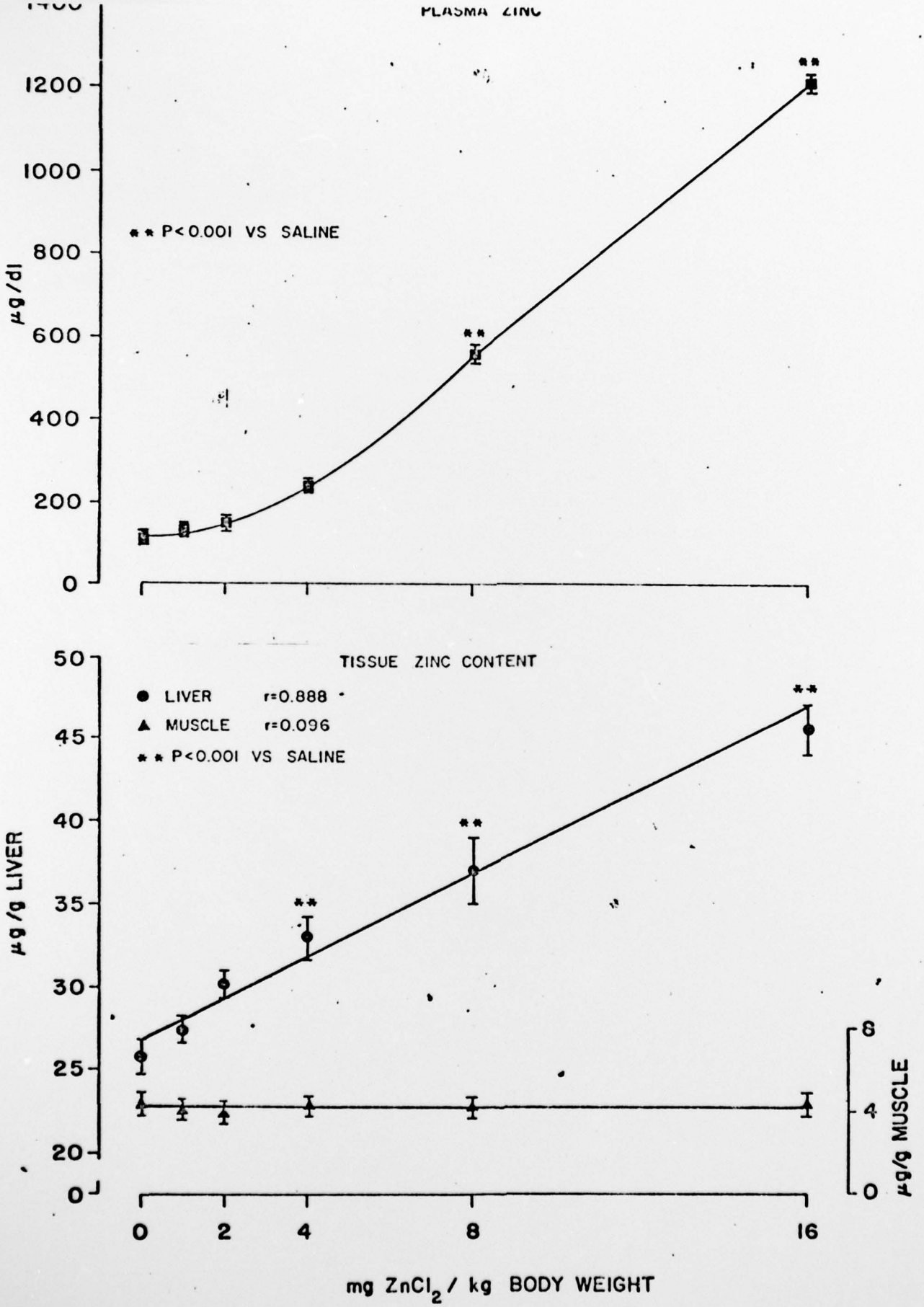
LEGENDS TO FIGURES

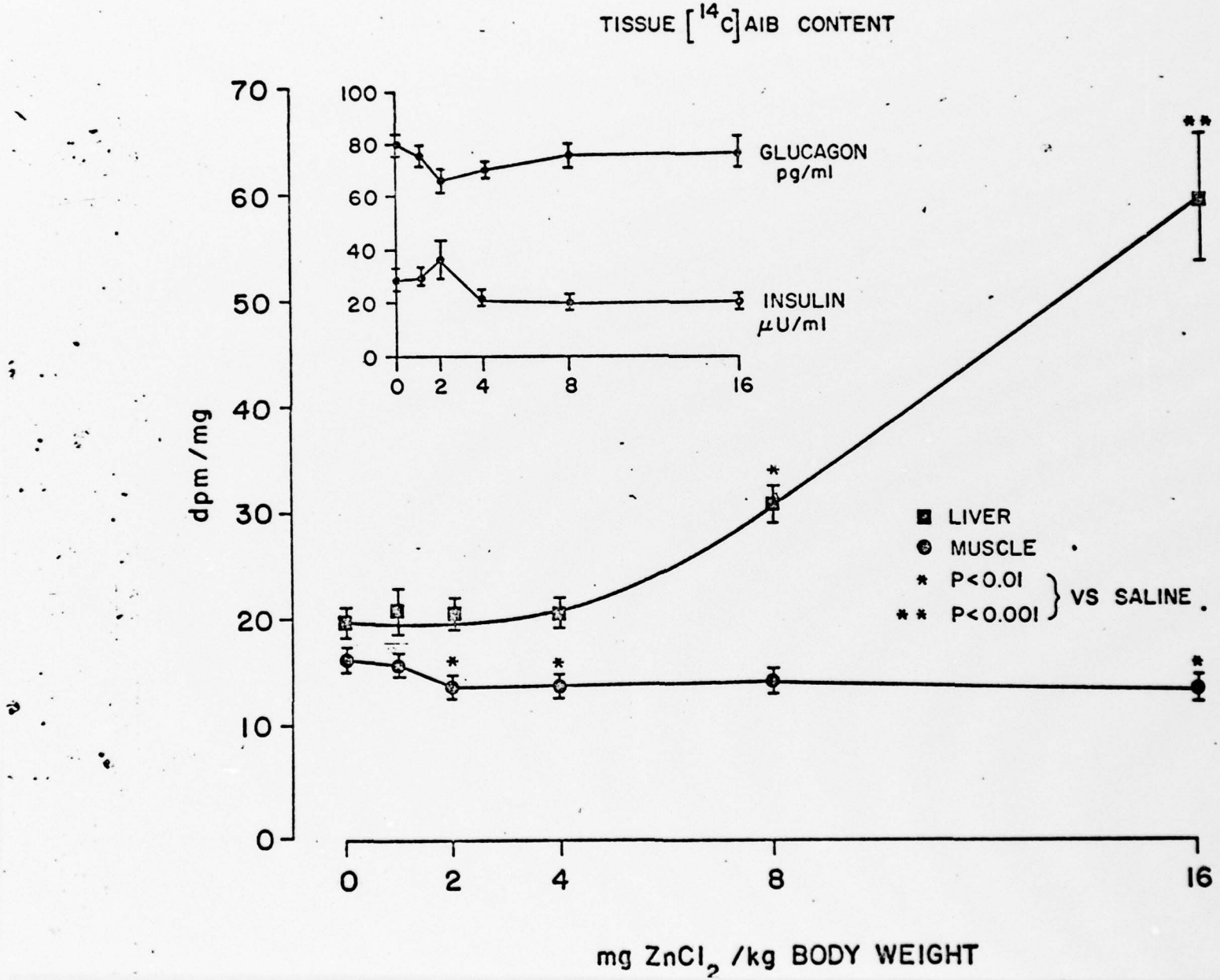
Figure 1a. - Plasma zinc concentration.

1b. - Liver and skeletal muscle zinc concentration.

Figure 2. - [^{14}C]Aminoisobutyrate content in liver and skeletal muscle.

Insert: Plasma glucagon and insulin concentrations.


PLASMA ZINC
1200
1000
** P<0.001 VS SALINE
800
µg/dl
600
400
200
0
**
**
TISSUE ZINC CONTENT
50
LIVER r=0.888
MUSCLE r=0.096
** P<0.001 VS SALINE
45
40
35
30
25
20
0
µg/g LIVER
**
**
**
6
4
0
µg/g MUSCLE
0
2
4
8
16
mg ZnCl2 / kg BODY WEIGHT


TISSUE [14C]AIB CONTENT
dpm/mg
70
60
50
40
30
20
10
0
100
80
60
40
20
0
GLUCAGON pg/ml
INSULIN μU/ml
0
2
4
8
16
LIVER
MUSCLE
* P<0.01
** P<0.001
VS SALINE
mg ZnCl2 /kg BODY WEIGHT

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